Nesting in close quarters:
Causes and benefits of high density nesting in painted turtles

By

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Abstract

Nesting is a costly time for female turtles, both energetically and from threat of predation. Females must ensure maximum survival of offspring for population stability and individual fitness. I observed signs of communal nesting in female Painted Turtles (*Chrysemys picta*). My goals were to determine; are females choosing to nest at high nest-densities, what cues do they use to select nest sites, are offspring benefitted. Using ArcGIS, I found that females nested in clusters, the location of clusters varied among years, and that nest site selection was not strongly determined by environmental characteristics. When female turtle models were placed on the nesting embankment females nested most often with the highest density of models. In ~25% of cases, nests were so clustered that eggs were deposited directly into existing nests or directly beside existing nests. Survival of clustered nests (49%) was higher than that of solitary nests (39%). In incubators, older clutches had faster incubation times, suggesting embryonic communication as a mechanism promoting hatching synchrony. We strongly suggest that female Painted Turtles choose to nest in close proximity to conspecifics, and that this clustering results in a fitness benefit.

Keywords

Communal nesting, egg clustering, nest synchrony, hatchling communication, synchronized emergence, embryonic development, nest behaviour

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Preface

Turtle populations can tolerate high levels of egg and hatchling mortality while maintaining stable populations, so long as the survivorship of adults is high enough to offset low recruitment (Stearns 1976). Adult female turtles are therefore expected to conserve energy for future reproduction (i.e., iteroparity), rather than investing into ensuring the present year's offspring will survive (Harms et al. 2005). Nest predation rates are also often high (Oddie et al. 2015), especially in environments with an increased abundance of predators (Ner and Burke 2008), so having reduced energy investment into current reproduction, including parental care, can be beneficial to female fitness (Spencer and Thompson 2003).

Nest destruction by predators is a common occurrence for most species of turtles. Turtle nests are unguarded, so using olfactory, tactile, or visual cues enables predators to easily find nests, even if the nests are hidden (Oddie et al. 2015). The eggs are packed with an energy rich yolk, creating a high energy meal for low investment (Wilhoft 1986; Lovich et al. 2018). In addition, turtle nests are a seasonally available, high density food source. Seasonal migrations of animals to feed on turtle nests have been observed (Tucker et al. 2007; Kell et al. unpublished, 2017). Turtle hatchlings also have a wide range of predators. Small mammals such as American red squirrels (*Tamiasciurus hudsonicus*) or eastern chipmunks (*Tamias striatus*) (Snow 1982), birds including many species of corvids (*Crovidae*) and herons (*Ardeidae*) (Rollinson and Brooks 2007), mesopredators such as raccoons (*Procyon loto*), striped skunks (*Mephitis mephitis*), and red foxes (*Vulpes vulpes*) that cause the most harm to nests (Wirsing et al. 2012; Spencer 2002), and larger predators

like Eastern wolves (*Canis lycaon*), and American black bears (*Ursus americanus*) (Lovich et al. 2014) also predate on nests and hatchlings. It is obvious that turtle nests face a variety of harmful factors, so it is expected that females would try to minimize these threats, while still keeping themselves safe.

One way turtles are thought to reduce threats to themselves during nesting is by displaying nest fidelity to a particular nesting area(s). Nest-site fidelity is the tendency for an individual female to return repeatedly to a given nest location or microhabitat (Rowe et al. 2005; Janzen and Morjan 2001). Sea turtles show nest-site fidelity to the original beach on which they hatched (i.e., philopatry; Avens and Braun-McNeill 2003). Nest-site fidelity in freshwater turtles has been observed in many species (Ernst and Lovich 2009), but does not usually occur for all individuals in a population; up to 40% (Spencer and Thompson 2003) of the population will return to the same site or location to nest (Christens and Bider 1987; Rowe et al. 2005). Most studies show that females in a population will display various levels of fidelity, some very strong and some very weak or not at all. Some individuals may show nest-site fidelity to a particular combination of environmental characteristics such as vegetation cover (Valenzuela and Janzen 2001), water content, and substrate (Rasmussen and Litzgus 2010). Many of these are the same characteristics that females select to increase the development, and therefore fitness, of their offspring. By demarcating a nesting area (230 m long) into 23 grid sections, each 10 m^2, I previously found (Kell 2016) that female painted turtles (*Chrysemys picta*) in Algonquin Park, Ontario returned to the same grid section in 47% of years, and the same 30 m x10 m section (3 consecutive grid squares) in 60% of years over a 5-year period. These are high fidelity values for small nesting areas and illustrate that

painted turtles return to a similar location each year even though there is opportunity to choose other locations, but fidelity is still low enough to change location if needed.

Females may show nest-site fidelity to reduce time on land, and therefore exposure to predators. This seems like a plausible explanation, but attempts to quantify the search time (time required to search out and execute nesting) through radio telemetry have failed to show support for this theory (Rowe et al. 2005; Iverson et al. unpublished). Using a novel technique to quantify search time, I found (Kell 2016) that search time increased with maternal age and body size, suggesting that as a turtle gets older, she is investing more energy in the nesting process. Searching for a nesting site can take a couple hours, up to days, and therefore requires a significant investment of energy (Congdon and Gatten 1989). Nest-searching behaviour by painted turtles therefore supports that females increase maternal investment over time.

Female turtles may actually invest more energy in their offspring than once thought, especially with the recent work on putative parental care in chelonians (Ferrara et al. 2013). As mentioned, the yolk of turtle eggs is very high in energy content (Wilhoft 1986), and this energetic commitment has been considered a major form of parental care by female turtles (Harms et al. 2005). The energy content dedicated to the yolk by the female is so great that somatic growth is significantly lowered after reaching maturity, resulting in reallocation of what is thought to be a significant portion of the female's energy intake (Harms et al. 2005; Hughes and Brooks 2006). Given the number of eggs a turtle may potentially lay over her lifetime, the amount of energy invested into the eggs is significant. It has also been clearly shown that turtles increase reproductive output (e.g. egg size, clutch size) as they age, resulting in increased maternal investment (Congdon and Tinkle 1983; Samson 2003).

Hughes and Brooks (2006) also predicted that older females may spend more energy on reproduction by being choosier about their nest sites than younger turtles, choosing sites that improve offspring survival to a greater degree.

Nest site selection is another form of parental care displayed by female turtles. Hughes and Brooks (2006) tested whether female painted turtles selected nesting sites based on certain characteristics and found that offspring survival was higher in areas chosen by females, compared to in random locations on the nesting area. They found that canopy cover, vegetation, and aspect ratio were most important in increasing offspring survival. Kolbe and Janzen (2001) also found that nest-site microhabitat characteristics (decreased vegetation cover and increased slope) were important for both survival and behaviour of hatchlings, suggesting that maternal behaviour such as nest-site selection, contributes to offspring survival. These characteristics and others such as substrate porosity, allow the eggs to develop at the optimal temperatures and humidity. The locations that females choose are important to the offspring's development (Wilson 1998), especially in the north where amount of heat units/day are limited. Open canopy allows for longer periods of sunlight, giving the nest more heat units so that the eggs can fully develop and hatch before cool fall temperatures (Janzen 1994). Schwarzkoff and Brooks (1987) tested the influence of nest-site selection on nest temperature in painted turtles and found that females chose nests that were significantly warmer and more likely to occur on slopes compared to random locations. In Algonquin Park, where it is considered part of the northern range limit of the painted turtle, Schwarzkopf and Brooks (1985) found that nest temperatures were more dependent on yearly weather variation than variation in site characteristics, suggesting that females select nest sites to maximize the probability that eggs will complete development. Christens and Bider

(1986) showed that nest placement may affect hatchling success, and Janzen (1992) showed that it may also affect locomotor abilities. All of these reasons make it evident that nest placement influences hatchling fitness, thus, natural selection should favour nest site selection that maximizes offspring fitness.

Females may choose the location of their nest in response to threat of predation. Low adult mortality is crucial to sustaining turtle populations (Enneson and Litzgus 2008), so ensuring that a female survives her nesting excursion is very important. If a female nests near her aquatic home range, then newly-emerged hatchlings will have easy access to aquatic habitat (Wilson et al. 1999), hatchlings will have reduced probability of predation during overland travel (Janzen et al. 2000), and the time that adult females are susceptible to terrestrial predators will be reduced (Congdon and Gatten 1989). However, high densities of nesting females near an aquatic habitat may result in increased nest predation because predators focus on concentrated food resources (Reitsma 1992; Robinson and Bider 1988; Marchand et al. 2002). Increased nest predation can cause females to travel farther from their "home wetland" to nest in an area with lower nest density and therefore lower predation risk (Spencer and Thompson 2003). However, if female survival is jeopardized while traveling to nesting sites far from a home wetland, females may choose to nest close to the water in areas of high nest predation risk, rather than risking their own lives and all future reproductive potential (Spencer and Thompson 2003). Some data suggest that as turtle nest density increases, so will predation rate, but there are other data showing that nest predation rates are density independent (Wirsing et al. 2012; Burke et al. 1998). Recent work in Algonquin Park found that very high nest density increases nest survival, potentially via prey saturation (Kell 2016). Reproductive synchrony refers to the tendency of individuals to carry out some stage

of reproductive cycle simultaneously with other members of the population (Ims 1990). Reproductive synchrony is a form of prey saturation as it increases numbers of prey above a level that predators can efficiently consume, resulting in a higher survival of adults and offspring (Sweeny and Vannote 1982). It is still debated whether nesting synchrony exists in freshwater turtles, but it has been observed in sea turtles (*Lepidochelys olivacea*) as large numbers of females nest within a short period of time and within a small area of available nesting beach (Eckrich and Owens 1995). Robinson and Bider (1988) found evidence for nesting synchrony in snapping turtles (*Chelydra serpentina*) as females tended to cluster, and synchronized nesting increased nest survival. Recently, synchronous nesting was observed during periods coinciding with first and second clutches laid by painted turtles in Algonquin Park (Kell 2016). These peaks in number of nesting females usually occur during or shortly after rainfall (Burke et al. 1998; Spencer and Thompson 2003; Kell 2016). Nesting peaks during rainfall may be a result of improved malleability of the soil, reduced importance for females to carry large amounts of liquid in their bladders, erasing scent that predators could cue in on, reduced risk of overheating, or as a trigger to initiate nesting synchrony.

If turtles are spending longer as they get older to search for a nest location, and they are searching for specific site characteristics, how is it that they find these locations? A recent study by Iverson et al. (2016) suggests that female painted turtles might use olfactory cues to locate nests, as they nested more often near nests scented with turtle urine than water. Observations of female turtles (e.g. painted turtle, snapping turtle (*Chelydra serpentina*), wood turtle (*Glyptemys insculpta*)) plowing their snouts into the ground while searching for a nesting spot have frequently been reported (Morjan and Valenzuela 2001, Kell personal observation) and may suggest that females are using olfactory cues to select nest sites.

Iverson et al. (2016) also reported that most females nest near other females or existing nests. Nest cluster behaviour has been seen, as mentioned above, but also to the extreme of females digging up nests of other females (Robinson and Bider 1988; Kell 2016; Moldowan personal observation). Iverson et al. (2016) suggests that this clustered nesting behaviour may be a form of predator satiation, saturation, or confusion, supporting Kell (2016) and Robinson and Bider (1988). Predator satiation or saturation is described as "safety in numbers" where prey occurs at high enough density that it lowers the probability of consumption of an individual by predators. Predator confusion still relies on a high density of prey, but instead of affecting consumption (handling time, stomach capacity, etc.), the large number of prey items confuses and overwhelms the predator thereby increasing the difficulty of targeting an individual prey. This is made even more interesting through my observation in Algonquin Park that female painted turtles often emerge onto land simultaneously to nest, and will flee together when faced with a threat, after one female abandons nesting. Communication between turtle embryos and adult turtles is becoming more widely studied and recognized (Spencer et al. 2001; Colbert et al. 2010; McGlashan et al. 2015). Multiple studies also now support that embryo communication and perception of stimuli is possible across the animal kingdom (Schwagmeyer et al. 1991; Warkentin and Calswell 2009; Aubret et al. 2016b). If communication in turtles is not as rare as once thought, then it could also be a key factor in explaining these nesting behaviors.

Painted Turtle

The painted turtle (*Chrysemys picta*, Schneider 1783) is a small freshwater turtle (12-14 cm shell length) native to much of the United States and southern parts on Canada (Ernst and Lovich 2009). Painted turtles occupy a wide range of habitats from lakes, ponds,

swamps, and creeks, preferring slow flowing water, a soft bottom, and abundant aquatic vegetation and basking sites. In Ontario, hibernation starts in late October, with turtles remaining dormant under ice until late April or early May (Ernst and Lovich 2009). Painted turtles will mate in the spring when aquatic vegetation is starting to emerge and feeding begins, but most mating occurs in the fall (Ernst and Lovich 2009; Moldowan unpublished, 2015). Painted turtles often move long distances, occasionally overland, from one water body to another or parallel to rivers and streams (MacCulloch and Secoy 1983). Males often disperse to new aquatic habitats in search of mates once they have attained sexual maturity (Ernst and Lovich 2009). Some reports from Saskatchewan report that males traveled 21.5-26 km, females traveled 7-8 km and juveniles <2 km between captures (MacCulloch and Secoy 1983). Female painted turtles in Algonquin Park can travel >1 km round trip, strictly overland through forest, to nesting sites (Brooks unpubl. data). In Ontario, females nest from late May to early July, with turtles laying up to two clutches a season, possibly three, averaging 5-12 eggs (Ernst and Lovich 2009). In Algonquin Park, 20% of females will lay a second clutch (Brooks unpubl. data). Females look for nesting areas free of vegetation and canopy cover with a loose substrate. Females may dig multiple test holes before completing their nest and often plow their head through or along the substrate before finding a spot to dig (Brooks unpubl. data; Kell 2016). In Canada, the painted turtle is as a species of Special Concern (COSEWIC 2018). Painted turtle sex is determined by TSD; producing females at high average incubation temperatures (30-32^{o}C), males at low average temperatures (22-26 ^{o}C), and both sexes at the pivotal temperature of 28 ^{o}C and at the lowest possible temperature for embryogenesis (20^{o}C) (Schwarzkopf and Brooks 1985).

Objectives

Although much research has been done on the nesting characteristics of turtles, recent work has uncovered some novel and truly interesting aspects of nesting behaviour and parental investment. Further research needs to investigate the causes and benefits of nest clustering behaviour, and painted turtles provide a perfect study organism to investigate this. In this thesis, I explored observations of communal nesting behaviours observed in painted turtles, and investigated what might drive these and what benefits a female may receive from nesting with other females. The goal of my study was to explore these observed nesting behaviours, to expand our knowledge of turtle reproductive biology and the recent literature of turtle parental care and communication.

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Nesting in close quarters: Causes and benefits of high density nesting in painted turtles

1.1 Introduction

High adult survival is crucial to turtle population stability (Congdon et al. 1987, 1993; Enneson and Litzgus 2008; Olofsson et al. 2009). High levels of egg and juvenile mortality, and low levels of recruitment, are tolerable, as long as adult females survive to invest energy into subsequent years of reproduction (Clutton-Brock 1984; Congdon et al. 1993; Harms et al. 2005). Nonetheless, females experience fitness benefits from increased offspring survival within a reproductive season, and from minimization of harm to themselves during nesting (Spencer 2002). Indeed, female turtles may invest more energy into their offspring than once thought; in addition to the energy-rich yolk (Wilhoft 1986), females select nesting sites that increase offspring survival (Hughes and Brooks 2006; Rasmussen and Litzgus 2010). Nest site characteristics such as canopy density, vegetation cover, aspect ratio, and slope are selected by females and significantly increase offspring survival (Janzen 1994; Kolbe and Janzen 2001; Hughes and Brooks 2006). These characteristics promote embryonic development (Wilson 1998), locomotor ability (Christens and Bider 1987), hatching success (Janzen and Morjan 2001), and ensure adequate heat-unit accumulation for incubation which can be limiting in the north and vary depending on yearly weather conditions (Schwarzkopf and Brooks 1985, 1987; Edge et al. 2017).

Turtles are generally considered to be slow, lumbering, non-social creatures (Wilson 1975), but this may not be the case at all (Davis 2009). Although there are still many unknowns about the social interactions of turtles, recent work has indicated that social interactions during nesting and hatching are more complex than once thought and communal nesting may be a conceivable phenomenon (Colbert et al. 2010; Ferrara et al. 2013; McKenna 2016). Turtle eggs are a widely exploited food resource (Snow 1982: Riley and

Litzgus 2014; Oddie et al. 2015; Lovich et al. 2018), and research suggests that turtles may partake in reproductive synchrony (Eckrich and Owens 1995), resulting in prey saturation to help reduce loss of offspring (Robinson and Bider 1988; Kell unpubl. data). Peaks in nesting activity often occur during rainfall events (Burke et al. 1998; Spencer and Thompson 2003) resulting in high density nest clustering. Iverson et al. (2016) even suggest that females may use olfaction to locate other female's nests, allowing nests to be deposited in close proximity to one another. Nest clustering may also allow embryonic communication among eggs and neighboring clutches (Spencer et al. 2001; Colbert et al. 2010; McGlashan et al. 2015), resulting in synchronous hatching which may also confer a survival advantage via prey saturation.

The aim of my study was to examine causes and benefits of nest site choice by female painted turtles (*Chrysemys picta marginata*) in a population where nest-site selection, reproductive synchrony, and nest clustering have been observed. The objectives of my study were to expand on the novelty of turtle social behaviour during nesting by: 1) quantifying whether female painted turtles nest in non-random spatial clusters, and in temporal clusters through nesting synchrony, 2) investigating how female painted turtles choose their nesting location, considering visual and olfactory cues from other females, and abiotic site characteristics, and 3) determining what benefits female painted turtles may receive from nesting in high density groups by investigating hatchling fitness through, 3a) nest survival, and 3b) hatchling survival via hatching synchrony through the mechanism of hatchling communication.

1.2 Methods

Study Site Description

My study took place in Algonquin Provincial Park, Ontario at Wolf Howl Pond (WHP) (45°34’40’’N, 78°41’22’’W) (Figure 1), a black spruce (*Picea mariana*) bog bisected by a decommissioned gravel and sand railway embankment (built ca. 1895), now part of the Mizzy Lake Hiking Trail. The embankment is 230 m long, and has a flat top (6-7 m wide) with minimal vegetation. The northeast side is shaded by mature trees (white pine, *Pinus strobus*, balsam fir, *Abies balsamea*, and tamarack, *Larix laricina*), and the southwest side is sloped (ca. 30^{o}). The southwest side of the embankment is 3-4 m wide and is the main nesting site for turtles at WHP. The embankment is also divided into 23, 10-m^2 grid sections (Figure 2), established in 1985-2008 (Schwarzkopf and Brooks 1985) and recreated in 2015-2017. The embankment is used annually as an oviposition site by 100-150 painted turtles.

Figure 1. Locations of all field sites used or sampled during the 2017 nesting season. Blue symbols represent the main wetlands that were sampled and monitored during nesting season and are part of the long-term turtle study (Est. 1978). Wolf Howl Pond (WHP) is the main site where most experiments took place. Green symbols represent sites that were used for the scent saturation experiment, allowing access to snapping turtle (*Chelydra serpentina*) nests and a sandy location for artificial nests.

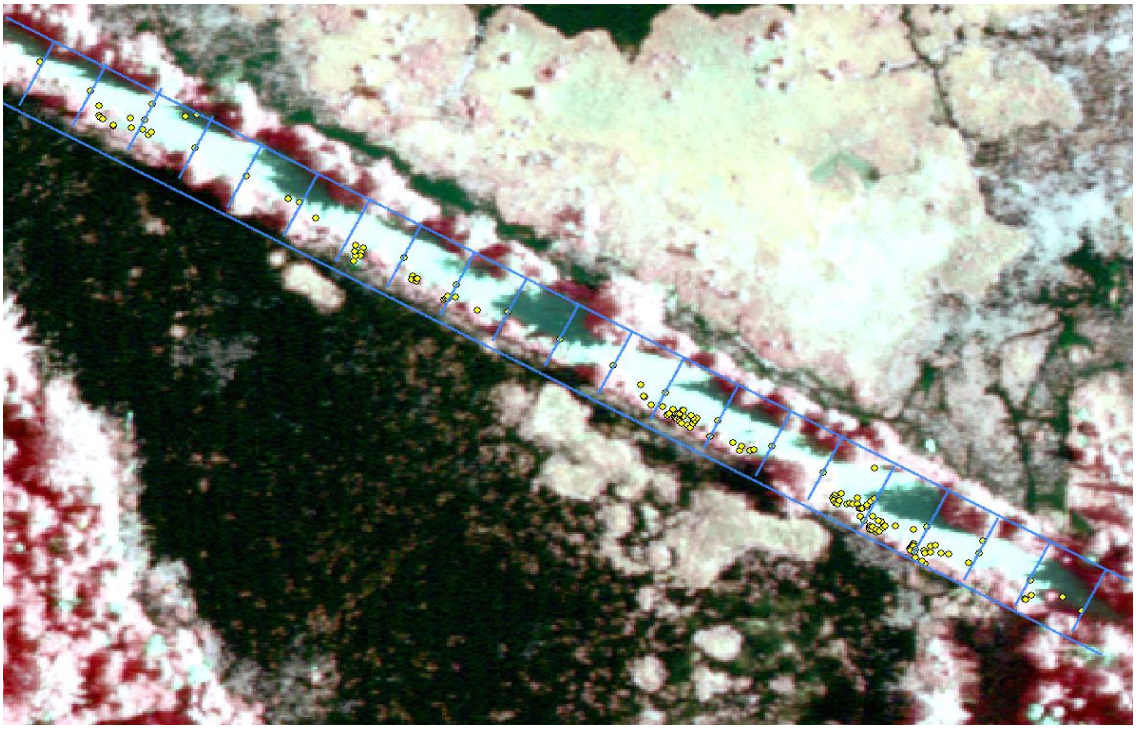

Figure 2. Decommissioned railway embankment bisecting Wolf Howl Pond, Algonquin Park where ~130-150 painted turtles (*Chrysemys picta*) nest each season. Embankment is divided into 23 grid sections. Yellow dots represent painted turtle nests from 2015 season as an example of nest distribution.

Data Collection

Nest location data from 2015, 2016 and 2017 were used to generate nest densities across years but all experiments took place during the 2017 nesting season. Nesting season varies slightly year to year but usually starts within the first week of June and runs until the end of June/start of July, lasting 3-4 weeks. The 2017 nesting season had a late start (June 14) and ran into the second week of July; however, nesting embankment monitoring stopped on July 7th due to other data collection priorities as nesting rates had dropped between 0-2 turtles per day. Throughout spring, we palpated the hind leg pockets of females, feeling for shelled eggs to mark the start of nesting season and the start of embankment monitoring. Embankment monitoring consisted of arriving each day between 1200-1400 h to start patrols before turtles began nesting, and walking the embankment back and forth at relatively consistent intervals (~15-20 min) until dawn when turtles remained non-terrestrial. Each nest was marked with a flagged stake (see below). At the start of each field season in 2015, 2016 and 2017, we raked the entire nesting embankment to remove any vegetation, so that all soil was freshly and consistently turned across the embankment. Predated nests were marked on their stakes with the date, and holes were re-filled with substrate, and the egg shells were removed and discarded. Any invested test pits were also re-filled at the end of the each day to reduce confusion by researchers during nest detection. An invested test pit (ITP) was defined as an attempted nest for which a female dug a partial nest chamber and then left for an unknown reason without laying eggs.

Clustering

To test whether nests were spatially clustered non-randomly, I used ArcGIS to conduct a nearest neighbor analysis. The location of nests at the end of each season were

recorded using a high accuracy Tremble GPS (accuracy ~30 cm). Nests were marked using 50 cm pigtail stakes with flagging tape labeled with nest ID and lay date. Stakes were located at the stake's length up the bank so that predators could not use the stakes as cues to locate nests (Rollinson and Brooks 2007). I compared the nearest neighbor distances among nests within each year (2015, 2016, 2017) to determine the level of clustering relative to random nest distributions, and I visually compared the distribution of nests between years by overlaying maps created in ArcGIS. I compared nest distributions among years to test whether females were choosing nest sites based on individual site fidelity (Rowe et al. 2005), and to test whether females chose nest sites based on where other females nested that year. Nearest neighbor analyses were completed with an edge effect strategy by tiling the data. Tiling the data subdivides the data points into gridded sections, increases accuracy by taking into account smaller areas rather than the entire distribution of points, which can bias the results when points are already in large groups or along linear distributions (Zhang et al. 2004).

I also wanted to know if painted turtles were participating in nesting synchrony by temporally clustering their nesting behaviour within a short time window. In each study year, there are generally 1-2 peaks in nesting activity that result in a large majority of turtles nesting within 1-3 days, with the first larger peak usually consisting of first clutches, and the second smaller peak consisting of late first clutches and early second clutches (Brooks, unpubl. data). The number of nests per day in each field season were visualized graphically to examine the possibility of nesting synchrony (Figure 3).

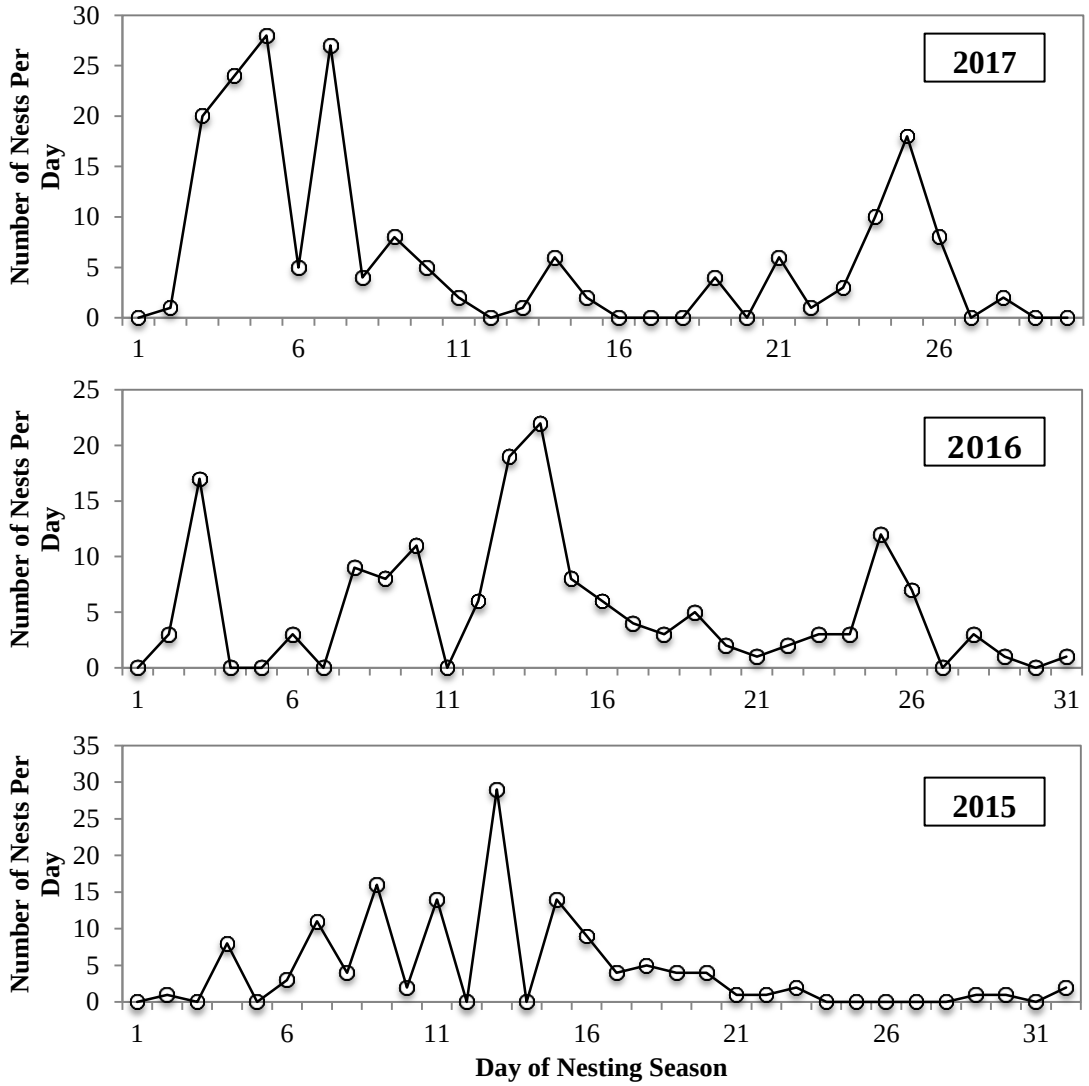

Figure 3. Total number of painted turtle (*Chrysemys picta*) nests laid per day over the course of the nesting season at Wolf Howl Pond (WHP), Algonquin Park in 2017 (top), 2016 (middle), and 2015 (bottom).

Olfactory Cues

To determine if females use olfactory cues to select nest sites, I constructed artificial nests scented with turtle urine or water. Female turtles hold urine in their bladders, which they release while digging nests (Iverson et al. 2016). As soon as female painted turtles were observed staging at the water's edge and moving onto land to search for nest sites, turtles were collected. We collected urine from all females that came up to nest on the first day of the nesting season by placing them in a clean container immediately after capture until they released their urine. Urine from all females (n=23) was mixed together (~800 ml) to reduce any maternal effects. No females were allowed to nest on the first day of the nesting season. At the start of the following day (second day of nesting season), 16 locations spaced >1 m apart, were selected in the five most popular nesting areas (based on long-term study; Brooks, unpubl. data) and based on site characteristics described by Hughes and Brooks (2006), so that artificial nests could be created in locations in which turtles would likely nest. In each of these locations, a pair of artificial nests was constructed 1 m apart (Figure 4). Each nest in the pair was then randomly assigned to one of two treatments: urine or distilled water (control). Each nest received 50 ml of liquid; 25 ml into the excavated cavity and 25 ml onto the substrate used to refill the cavity (no eggs were present in these artificial nests). Nests were dug using a spoon by researchers wearing latex gloves to prevent transfer of human scent. These artificial nests were the only "nests" present at WHP when females re-initiated nesting on the second day of the nesting season.

I examined whether females chose to nest more often near the urine or distilled water scented nests by counting the number of nests within a 0.5 m radius circle of each treatment. I applied these counts to all nests laid over the course of the nesting season, and to nests laid

within only the first 2 days of artificial nest creation. I examined these two time periods because I did not know if the artificial nests would be detectable by females for the whole season, or only for the first couple days until many other real nests were laid, obscuring the artificially-scented nests. I also recorded all instances (nests or invested test pits (ITP)) in which females nested on top of or touching another nest, nest cavity, or recently predated nest. Two Pearson's Chi-square tests of Independence, one for the entire season and one for within 2 days of artificial nest creation, were used to compare the number of real nests laid within a 0.5 m radius circle of each artificial nest treatment (urine or distilled water). Tests were performed in R v 3.3.1 GUI using contingency tables in package *Rcmdr*.

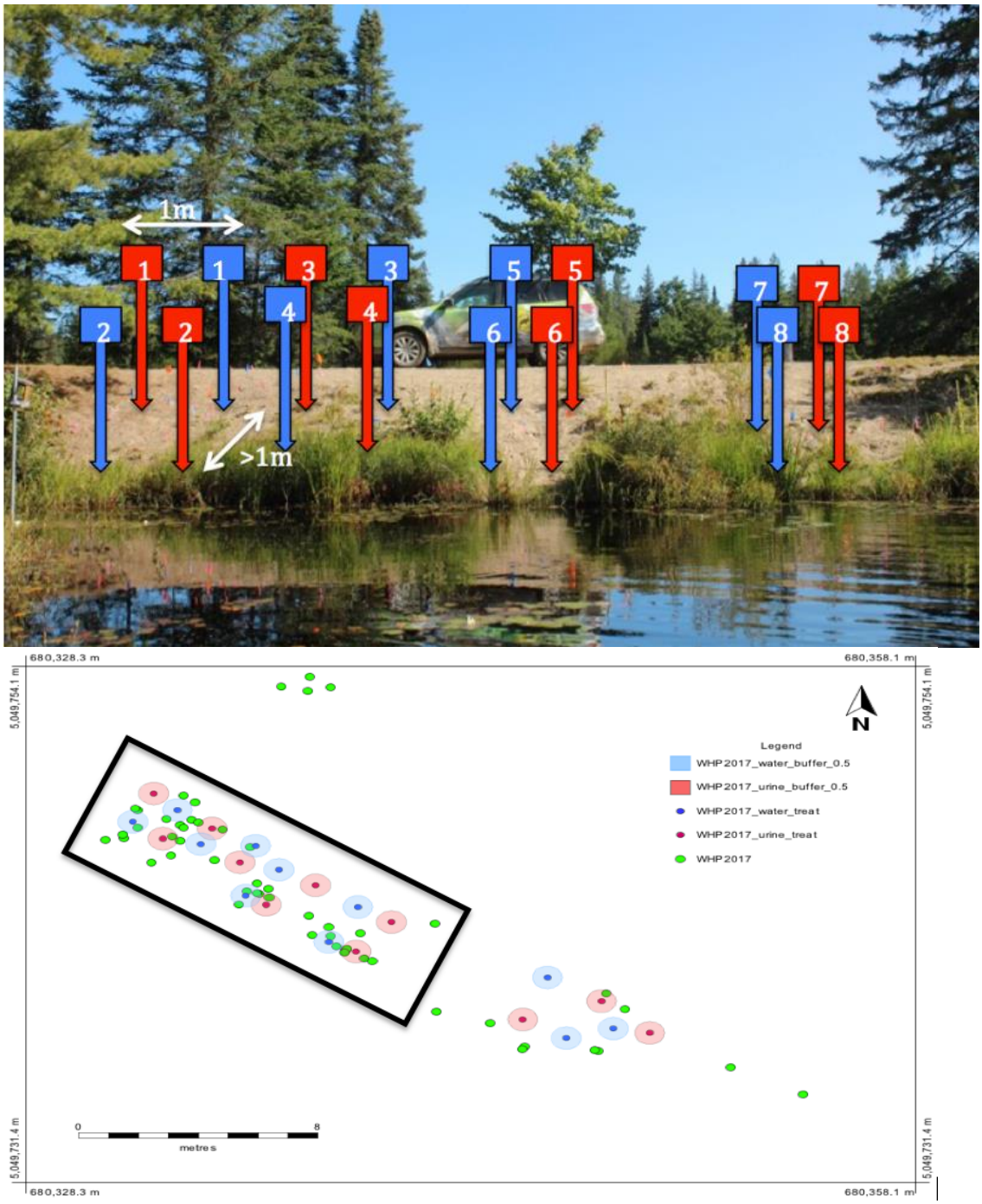

Figure 4. (Top) Illustration of layout of 8/16 scented artificial nest pairs created at the start of nesting season at Wolf Howl Pond (WHP), Algonquin Park. Red markers refer to urine-scented nests and blue markers refer to distilled water-scented nests. Numbers on the markers correspond with nest pair. (Bottom) Layout of 11/16 nest pairs at WHP with distribution of natural nest locations in 2017 (green dots). Red circles refer to the urine treatment and blue

circles refer to the distilled water treatment. The black box shows the area of the embankment shown in the above photo.

Visual Cues

Turtle models were used to determine if females use visual cues from other females to choose nesting locations. Turtle models were carved out of Styrofoam, coated in a layer of tile adhesive and burlap, and painted to resemble a nesting female (Figure 5). For this experiment, three areas at WHP were chosen based on their annually high numbers of nesting females (Brooks, unpubl. data) and their relative lack of vegetation. Vegetation at the water's edge was cut in all 3 locations so females in the water would easily see the embankment and models. Models were set and monitored near the end of nesting season (June 30 - July 7), after the olfaction experiment was complete, and after the first large peak in nesting. There were three treatments: control (no models), low density of females (1 model), and high density of females (3 models). On a given day, treatments were randomly assigned to one of the three locations. Treatments were at least 8 m apart. At the end of each nesting day, the number of nests and the number of invested test pits (ITP) were counted within ~2.5-3 m of models. This distance was used as it was the average distance from the models to the edge of each location's vegetation (>1 m in height). The models were rotated among the three locations each day, allowing each treatment to be in each area three times. I examined whether females nested more often with other females already present (i.e., the models) or by themselves. Three Pearson's Chi-square tests of Independence were used to determine if there were differences in number of nests laid, number of ITPs dug, and number of nests + ITPs among high density, low density, and control treatments. Tests were performed in R v 3.3.1 GUI using contingency tables in package *Rcmdr*.

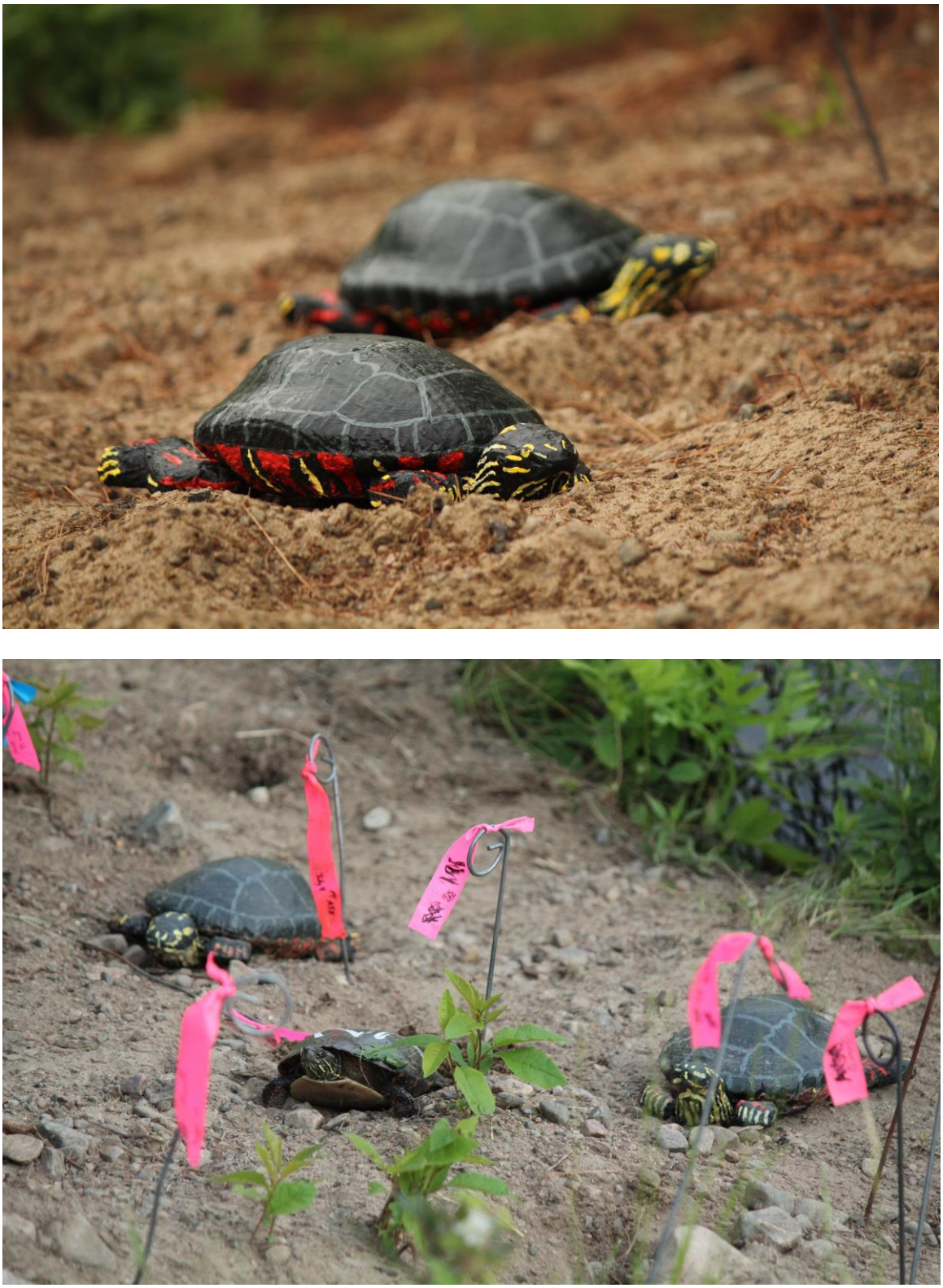

Figure 5. (Top) Female turtle models on the Wolf Howl Pond (WHP), Algonquin Park nesting embankment during the 2017 nesting season. Three different model densities (i.e.,

high-3 models, low-1 model, control-no models) were positioned at WHP to test the influence of visual cues from other females in nesting preference. Models were created from a combination of shaped foam covered in burlap and tile adhesive, then painted. (Bottom) Female painted turtle nesting amongst the turtle models.

Environmental Conditions

To test if females choose nest sites based on site characteristics, multiple environmental characters were measured for each nest. At the time the nest was laid, soil, air and water temperatures were measured. At the end of the nesting season, after all nests had been laid, a suite of characteristics that tend to remain fairly consistent or unchanging throughout nesting season was also measured, including: understory vegetation density, overstory vegetation density, soil temperature at noon, and slope. Soil temperature at noon was taken as an indication of heat retention along the embankment. Soil moisture, substrate type, and aspect ratio were not measured for each nest, as the embankment is an old linear railway bed constructed from sand, thus all nests point in the same cardinal direction and have consistently the same substrate. Soil density was also controlled for by raking the entire embankment prior to the nesting season (as noted above). Overstory density was measured using a spherical densitometer at breast height for the four cardinal directions, then averaged. Understory density was measured by placing a 1 m x 1 m grid (containing 100 squares) centered on the nest and counting how many sections contained vegetation. All plants under 1 m height were considered understory vegetation. Slope was measured using a clinometer mounted to a piece of wood so slope could be measured at ground level.

Euclidean distances (a measure of density based on a nest's relative distance to all other nests) for each nest in the 2017 field season was created using ArcGIS by extracting raster data. Linear regressions were then run between all abiotic characteristics and Euclidean distance to determine the effect of abiotic condition on nest density. A Principle Component Analysis (PCA) was used to visualize the interactions among all abiotic characteristics in R v 3.3.1 GUI.

Nest Predation and Survival

To determine if nest clustering affects nest predation, I examined predation rates of both natural (WHP) and artificial nests (created at Sand Dunes). Over a month after the end of the nesting season and nearing the end of summer (Aug 28th), I did a final check of nests at WHP to determine predation status. All nests at WHP were grouped into one of two categories: solitary (>20 cm from another nest) or clustered (<20 cm from another nest). A distance of 20 cm was used, as this is the minimum distance a female can nest beside another nesting female without touching and interfering with her during the nesting process. The predation status of nests was recorded and compared between the two groups. Nests were not monitored after this point or dug up to determine success rate.

I also did a manipulation experiment at Sand Dunes (45^{o}34'31"N, 78^{o}42'28"W), another nesting site that is part of the long-term study. Sand Dunes are a large open area (~30 m wide and 200 m long) about 2 km from WHP; it has few trees and the substrate is loose sand. I created two artificial nest densities: low (one solitary nest) and high (3 clustered nests). Each density was in a 0.5-m radius circle, with the solitary nest located in the middle and the clustered nests located in the middle but in a triangular pattern spaced 20 cm apart. Each nest was dug with a spoon 5-10 cm below ground and filled with 5 snapping turtle (*Chelydra serpentina*) eggs collected from turtles that nested at Joe Lake (45^{o}34'32"N, 78^{o}42'50"W; continuation of decommissioned railway embankment). Snapping turtle eggs were used because a large number of eggs could be collected from fewer adults compared to painted turtles (i.e., snapping turtles lay substantially larger maximum clutch sizes than painted turtles, 60 eggs vs. 14 eggs, respectively; Ernst and Lovich 2009). There were 15 nest pairs assembled, creating two parallel rows (A and B) with one density from each treatment

in each row. The 0.5-m radius circles were placed 2 m from their apposing density treatment in the opposite row and spaced 2 m from the neighboring pair. Densities were randomly assigned to row A or B. These nests were all created on the same day and monitored every 5 days up to 15 days after creation. At the end of the field season (Aug 28th), all nests were dug up to check final survival status. All nests were flagged 0.5 m from their circle to mark their locations and so that predators would not cue in on the flags (Rollinson and Brooks 2007). Any nest that was predated was left untouched, with sand and eggshells scattered around.

A Pearson's Chi-square test was used to compare survival between solitary and clustered nests at WHP and Sand Dunes. The expected predation frequency for all tests was based on the general predation rate (57.3%) from nests at WHP in the 2017 season. Each nest at WHP was considered their own treatment unit and recorded as predated or survived, but then additionally separated into solitary or clustered treatment groups based on the 20 cm spacing. Observed predation frequency was calculated from the actual survival of all clustered nests and survival of all solitary nests at WHP. At Sand Dunes, nest survival between clustered and solitary nests was also compared using expected predation frequencies from WHP and observed predation frequencies from clustered and solitary treatment groups at Sand Dunes. Tests were performed in R v 3.3.1 GUI using contingency tables of package *Rcmdr*.

Hatchling Communication

A manipulative experiment (modified from Hudson et al. unpubl. data) was conducted in 2017 to determine whether communication between eggs is possible, and if so, what form of communication is used. Clutches of at least 9 eggs were collected from female

painted turtles and processed (measured and weighed), and then assigned to incubation treatments in containers (see below and Figure 6) and incubated at a constant 30℃. Four days later, additional clutches of at least 9 eggs were collected and paired with the previously-collected clutches in their respective positions within containers (Figure 6). I ended up with 10 pairs of nests laid 4 days apart. Due to difficulty of collection, some early treatment nests were retained briefly at ~15℃ to stall development, to allow all experimental pairs to be 4 days apart in incubation period. Once a pair was established, clutches were separated into 7 containers (1 large and 6 small); 6 eggs from each clutch in a larger main container, and the remaining 3 eggs from each clutch in their own separate smaller containers (Figure 6). Only 9 eggs from each clutch were ever used and any remaining eggs were returned to where they were collected. The larger main container consisted of a middle section and 6 side compartments, divided with foam board. This set-up allowed 3 treatments, representing two possible forms of communication: mechanical, auditory and the control. The middle section of the large container was for mechanical communication, with 3 eggs from each clutch positioned side by side with their shells touching. The outer compartments of the large container were for auditory communication, holding one egg per compartment, with the foam walls blocking out any mechanical stimuli. The 3 smaller containers for each clutch were used for the controls, as eggs were isolated, thus preventing communication.

Containers were filled with a 1:1 Vermiculite: water mix to keep eggs moist. Containers and eggs were checked twice a week, rotating the containers between shelves within the incubator to maintain constant temperature and moistening containers to maintain humidity. After 45 days, eggs were checked every day to record pipping dates. Once turtles had pipped and hatched, hatchling carapace length, hatchling mass, and hatchling yolk sac

diameter were recorded. The number of days in which the hatchlings stayed in their eggs after pipping was also recorded. Once all eggs had hatched within a container, or were considered nonviable, hatchlings were moved to holding enclosures based on location of collection until release. To represent a natural nest chamber in which hatchlings remain with other offspring and eggs, hatchlings were left in containers until all eggs had hatched or were deemed nonviable. Eggs were not randomized among treatment containers so that my methods would be consistent with those of Hudson et al. (unpub. Data), to allow for future comparisons.

Before analysis, I explored the data for influential outliers and collinearity (Zuur et al. 2010). I used a Linear Mixed-effect Model (LMMs) fit by REML, correcting for random effects of maternity, collection location, and incubation container, then adjusted with Tukey's HST to determine if incubation duration, hatchling body condition, and yolk sac diameter differed between communication treatments and oviposition time (early vs late). Hatchling body condition was estimated using the residuals from a regression of hatchling mass (log) on body size (log carapace length) (Litzgus et al. 2008). Assumptions of normality and homogeneity of variance were met. Models were run in R v 3.3.1 GUI using functions *lme*, *lsmeans*, and *emmeans* using packages *nlme* and *emmeans*.

Figure 6. Container setup for the hatchling communication experiment. There are two clutches represented: clutch A (yellow circles) laid on day 1 and clutch B (red circles) laid 4 days after clutch A. Each clutch was separated into 3 treatments: mechanical communication (middle chamber of large main container, allowing eggs to touch), auditory communication (individual compartments of large main container separated by foam), and controls (individual smaller containers preventing any form of communication). Image shown without container lids on. All eggs were incubated at 30℃. $N_{clutches}$=20, N_{pairs}=10, N_{eggs}=9.

1.3 Results

Clustering

In each study year (2015, 2016, 2017), nests were significantly clustered (i.e., not randomly distributed) on the WHP embankment (Table 1, Figure 7). Clustering increased as the number of nests increased. More than 80% of nests were confined to about 40% of the WHP embankment (Figure 6), and this area is comprised of 6 nesting sections in the study grid (Figure 2). Examination of each of these sections reveals that there are differences in locations of nest clusters between years. For example, in sections 2 and 4 (Figure 7), in 2015 females nested at relatively low densities compared to 2016 and 2017, while the highest density was in section 6. In section 2, in 2017 females nested closer to the water and in 2016 nested farther up the embankment. In sections 3 and 5, in 2017 females nested to the east side whereas in 2016, they nested more to the west side. Along with the clustering on the embankment, turtles also appeared to cluster temporally with the majority of their nesting concentrated in two short time windows: 4 days in a larger peak at the start of the nesting season, and 3 days during a smaller peak at the end of the nesting season (Figure 3).

Table 1. Nearest neighbor (NN) analysis output examining probability of nest clustering by painted turtles (*Chrysemys picta*) in nesting seasons 2015, 2016 and 2017 in Algonquin Park. In all years, the z-score is < -1.96 indicating that nests were significantly clustered.

Statistic	2015	2016	2017
Observed Mean NN Distance	0.893	1.58	0.625
Expected Mean NN Distance	1.896	3.216	1.64
Standard Error	0.084	0.13	0.064
NN Ratio	0.471	0.491	0.381
z-score	-11.978	-12.579	-15.805
p-value	<0.0001	<0.0001	<0.0001

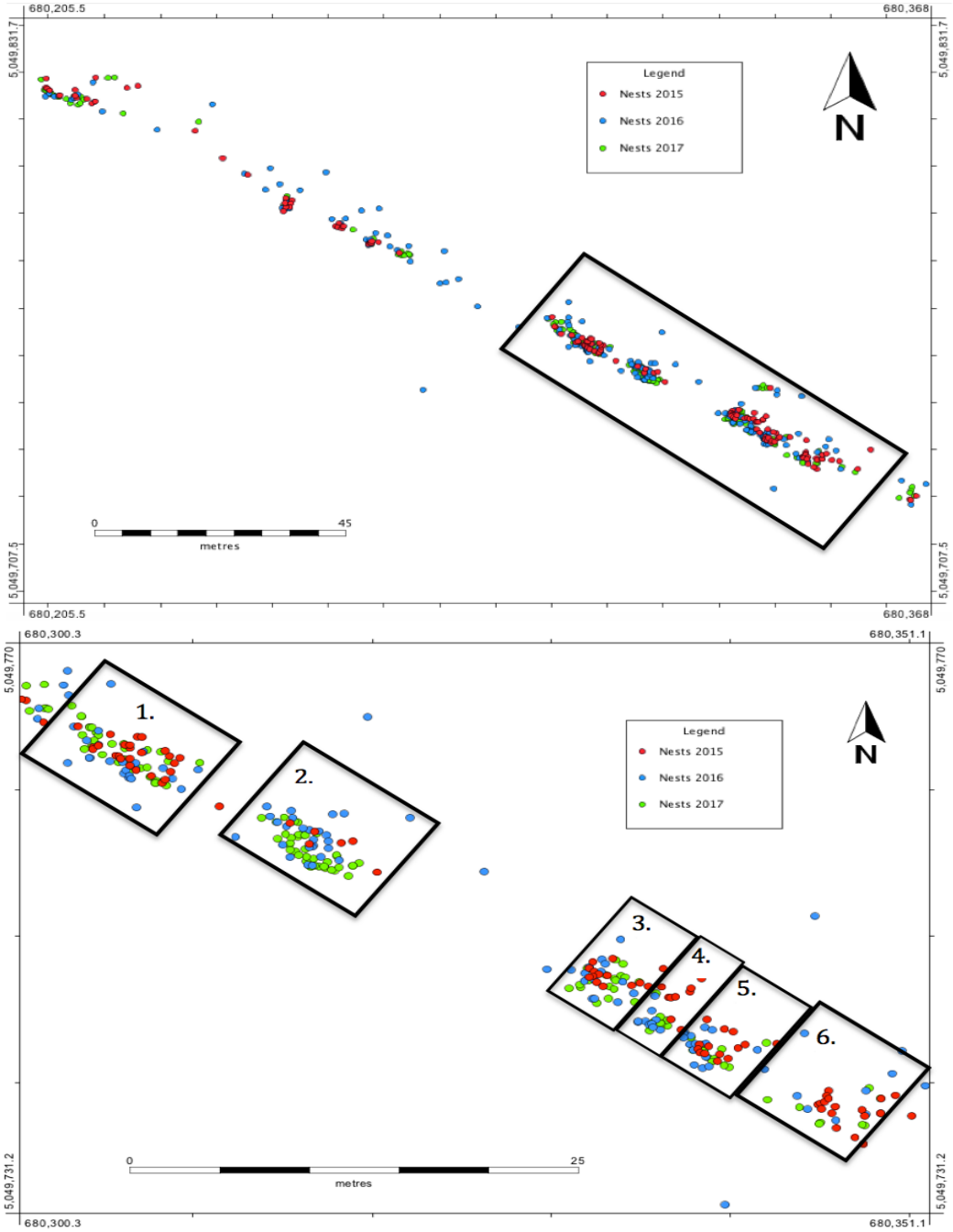

Figure 7. Maps of painted turtle nests at Wolf Howl Pond (WHP), Algonquin Park. (Top) All nests from the 2015, 2016, and 2017 nesting seasons. The black box represents the area shown in the zoomed-in map below. (Bottom) Nests from a section of WHP (~40%) which receives >80% of nests every year. Black boxes divide the nesting embankment into 6 major clusters, based on areas open of vegetation and most-used by turtles for nesting. These boxes facilitate visual comparison of nest clustering and nest numbers among the 3 years.

Olfactory Cues

The number of real nests within a 0.5 m radius of urine-scented artificial nests (N_{urine}=20, 1.25 ± 0.38) and distilled water-scented nests (N_{water}=18, 1.13 ± 0.36) did not differ (X^2=0.05, df=1, p=0.82). Of the 9 real nests laid within the first two days after artificial nests were constructed, again, the number of nests within a 0.5 m radius of urine-scented nests (N_{urine}=6, 0.38 ± 0.15) and distilled water-scented nests (N_{water}=3, 0.19 ± 0.1) did not differ (X^2=1.20, df=1, p=0.27), although there were twice as many nests near urine-scented nests. During the 2017 nesting season, 14 invested test pits were found on top of other nests or predated nests, and 27 nests were laid on top of another nest or predated nest. In 8 of 27 cases (30%), a female dug up another turtle's nest. Looking at the total nesting activity (invested test pits plus nests), 25% of the 165 nests recorded in 2017 at WHP had another nest or nest chamber constructed on top, or directly beside them, resulting in the nest chambers touching (Figure 8).

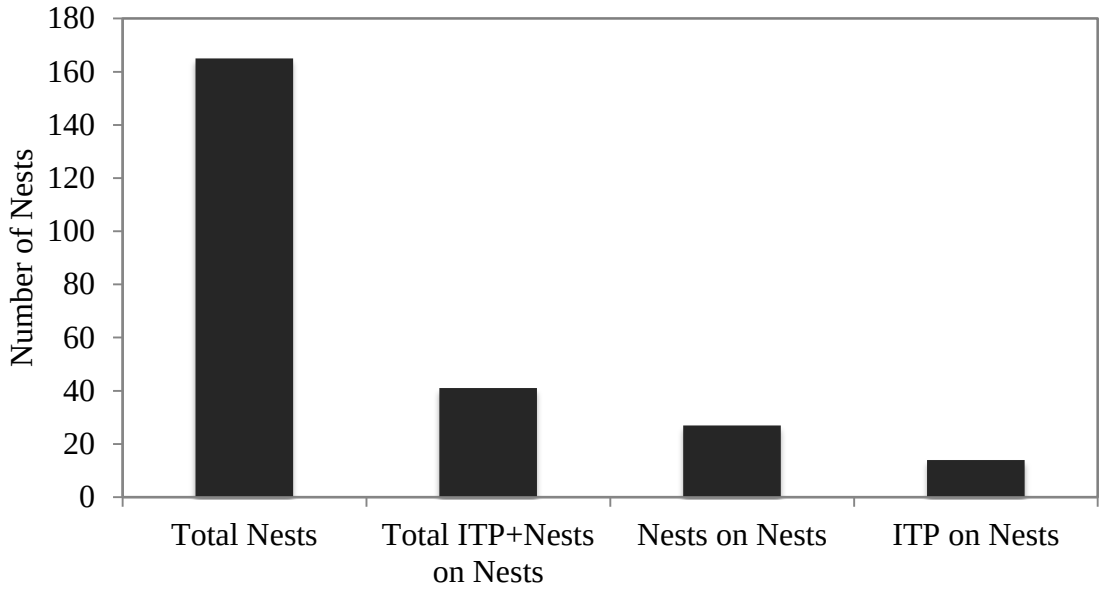

Figure 8. Nesting activity (invested test pits (ITP) and nests) observed on top or directly beside nests at Wolf Howl Pond (WHP), Algonquin Park during the 2017 nesting season. ITP consisted of digging until a chamber was semi-formed and then leaving without laying eggs. Nests consisted of chambers that contained eggs, or previously contained eggs that season and had been dug up either by another turtle or predator.

Visual Cues

Females displayed significantly more nesting activity near the turtle models (Figure 9). Over the 9 days of data collection, female turtles nested most often among models at high density (n=9 females), less often in the low density of models (n=5), and least often in the control (n=3). The number of invested test pits differed significantly among nest densities (high density=31, low density=20, control=9; X^2=6.55, df=2, p=0.04). Similarly, overall nesting activity (invested test pits plus nests) differed significantly among model densities (X^2=8.14, df=2, p=0.02), with the most nesting activity occurring with models at high density (n=40), moderate activity with models at low density (n=25), and the least activity in the control (n = 12).

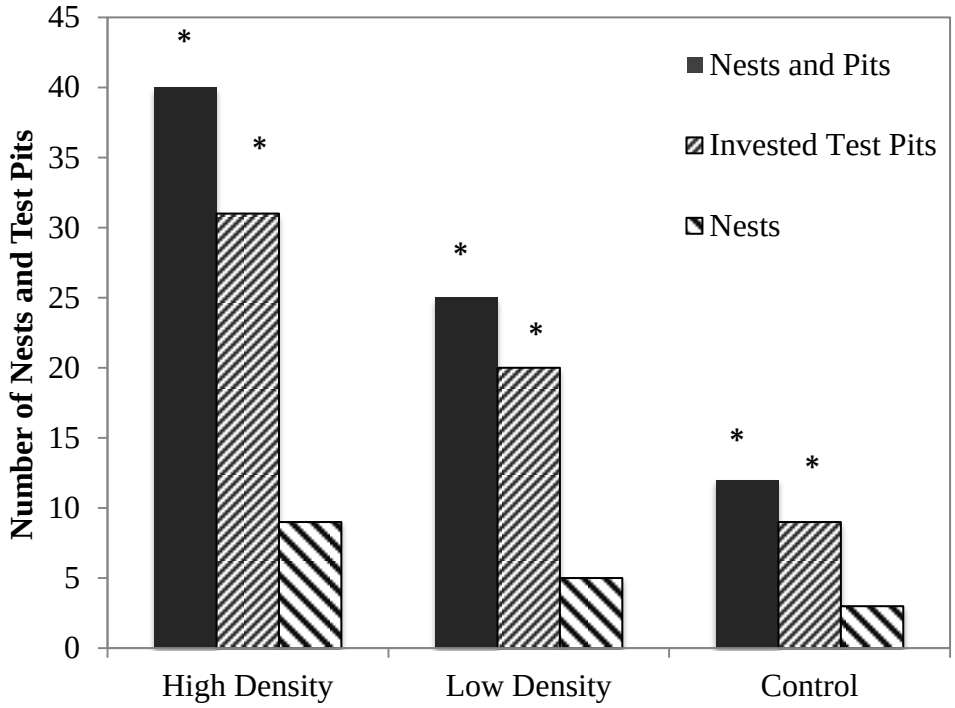

Figure 9. Nesting activity by female painted turtles (*Chrysemys picta*) at Wolf Howl Pond (WHP), Algonquin Park during the 2017 nesting season in response to different densities of model turtles. Densities consisted of 3 models (high), 1 model (low), and 0 models (control). I considered a nest as a cavity containing eggs, and an invested test pit (attempted nest) as a cavity close or fully dug but not containing eggs. Data were collected over a 9 day period near the end of nesting season, with each treatment in each location 3 times. There was a significant difference in the number of invested test pits among treatments, and in the number of invested test pits + nests among treatments, as indicated by the asterisks.

Environmental Conditions

Table 2 summarizes the relationships among the environmental characteristics and each nest's proximity to neighboring nests (Euclidean distances). Only understory vegetation ($F=6.25$, $R^2=0.029$, $p=0.013$) and slope ($F=4.32$, $R^2=0.019$, $p=0.039$) were significantly related to Euclidean distance. As embankment slope increased and understory vegetation decreased, the proximity to neighboring nests increased. From the PCA analysis, understory vegetation had large negative loadings on PC 1 and nesting day had large positive loadings on PC 2 (Figure 10). I found interactions among soil, air and water temperatures; slope and overstory density; and lastly, vegetation and overstory density.

Table 2. Relationships between abiotic characteristics and Euclidean distances for all nests on the embankment at Wolf Howl Pond in Algonquin Park during the 2017 field season. Understory vegetation and slope were significantly related to Euclidean distance (bold font). The nesting area is a linear, human-constructed embankment of sandy material (built ca. 1895), so cardinal direction, substrate type, and soil moisture were all consistent. Bold values indicate significant differences.

Characteristic	F-stat	DF	Adjusted R^2	p-value
Understory Vegetation	**6.248**	**1 and 172**	**0.029**	**0.013**
Over story Density	0.923	1 and 172	-0.0004	0.338
Slope	**4.315**	**1 and 172**	**0.019**	**0.039**
Nesting Day	0.305	1 and 173	-0.004	0.581
Air Temperature	1.563	1 and 164	0.0033	0.213
Soil Temperature	0.006	1 and 160	-0.0062	0.937
Water Temperature	0.4	1 and 139	-0.004	0.528
Clutch Size	1.142	1 and 165	0.0008	0.286
Soil Temperature (noon)	1.517	1 and 172	0.002	0.219

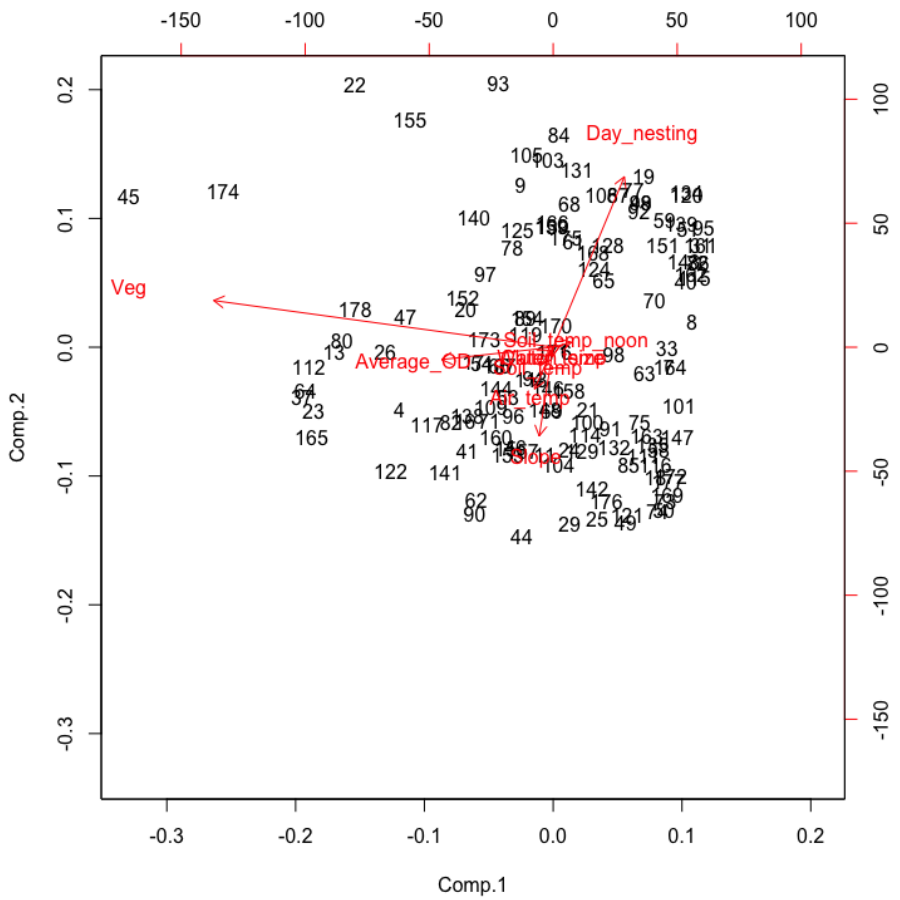

Figure 10. Principle component analysis conducted using the nest characteristics collected from painted turtle (*Chrysemys picta*) nests at Wolf Howl Pond (WHP), Algonquin Park during the 2017 nesting season. Understory vegetation had a strong loading with PC 1, and day of nesting season had strong a loading with PC 2 suggesting that understory vegetation and day of nesting season are the strongest drivers of nest number.

Nest Predation and Survival

At WHP, clustered nests had higher survivorship (49%) than solitary nests (39%; Figure 11), but the difference was not statistically significant (X^2=0.507, df=1, p= 0.476). Predators of nests at WHP consisted primarily of common ravens (*Corvus corax*) and some red fox (*Vulpes vulpes*) based on predation characteristics (corvids: small hole, no egg shells; fox: large hole, eggs shells scattered and still remaining).

At Sand Dunes, my manipulation experiment revealed that clustered nests (15.6% survivorship) had marginally higher survival than solitary nests (13.3%, Figure 12) but the difference between treatments was not significant (X^2=0.012, df=1, p=0.911). Considering each treatment unit's area as a location, the clustered treatment had marginally significantly more locations with un-predated nests left (clustered=7/15 (47% survivorship), solitary=2/15 (13% survivorship); X^2=3.65, df=1, p=0.058) All nests in the Sand Dunes experiment appeared to be predated by red foxes.

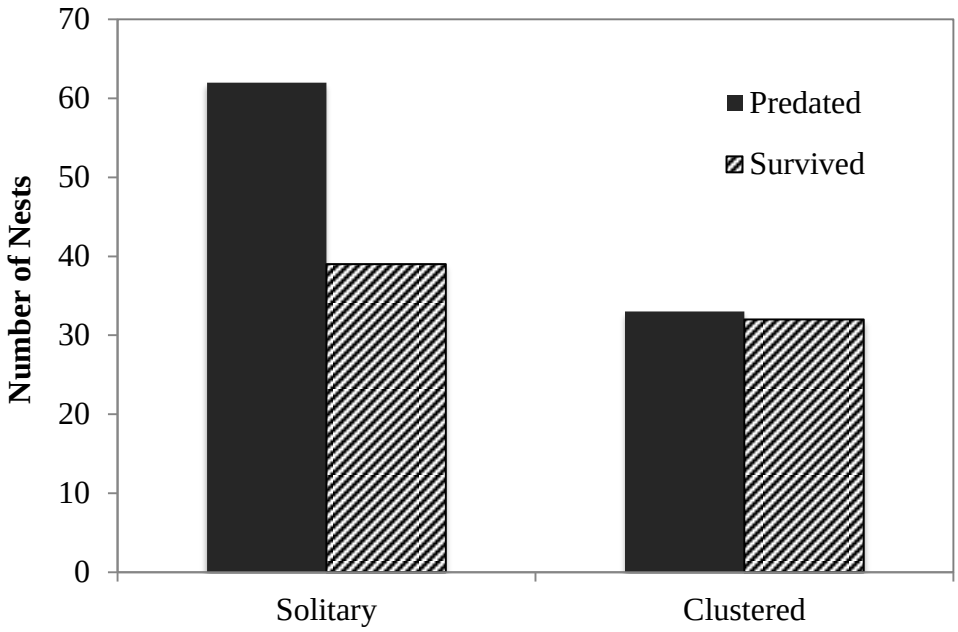

Figure 11. Level of relative predation for solitary (N=101, predated=61%, survived=39%) and clustered (N=65, predated=51%, survived= 49%) painted turtle (*Chrysemys picta*) nests at WHP in Algonquin Park from the 2017 nesting season. Nests were classified as clustered if they were within 20 cm of another nest. Nests within the clustered treatment had a ~10% higher relative survival compared to survivorship within solitary nests.

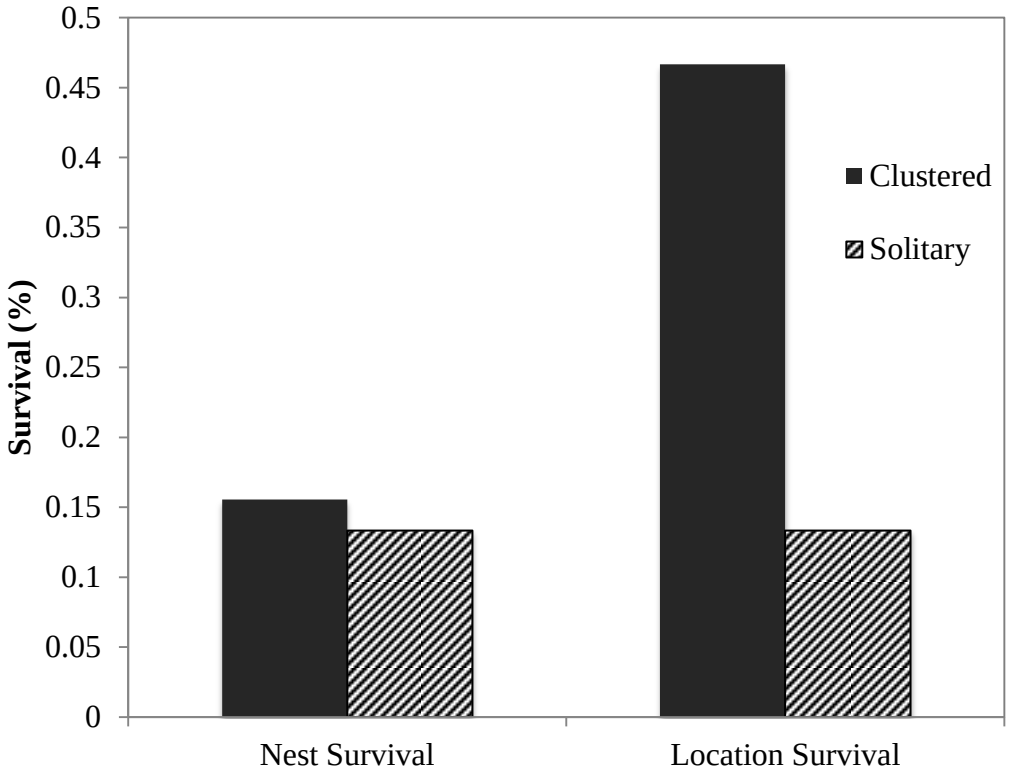

Figure 12. Nest survival for clustered and solitary nests artificially created with snapping turtle (*Chelydra serpentina*) eggs at the Sand Dunes in Algonquin Park. I examined individual nest survival and location survival (any nest remaining per location). Clustered nests were a group of 3 nests within 20 cm of each other, and solitary nests were a single nest. At the individual nest scale, survivorship did not differ between clustered and solitary nests, but at the location scale, survivorship was higher for clustered nests than solitary nests.

Hatchling Communication

Incubation duration of painted turtle eggs varied among communication (auditory, mechanical, control) and incubation treatments (early, late; Table 3, Figure 13). In all cases, the late treatments had shorter incubation durations than their early counterparts. The mechanical treatment for both early and late incubation had the shortest incubation durations. Mechanical-late had the shortest incubation duration compared to all other treatments and was significantly shorter than mechanical-early by 2.16 ± 0.62 days ($t=3.503$, $p=0.028$), auditory-early by 3.01 ± 0.61 days ($t=4.94$, $p=0.001$), auditory-late by 1.21 ± 0.39 days ($t=3.056$, $p=0.034$), and control-early by 2.7 ± 0.61 days ($t=4.45$, $p=0.0004$). Auditory-early was also significantly shorter than control-late by 1.99 ± 0.62 days ($t=3.24$, $p=0.046$). All other comparisons were not significant (overlapping 95% confidence intervals).

Yolk sac size and body condition of painted turtle hatchlings did not differ between all incubation treatments ($t=1.08$, $df=47$, $p=0.287$; $t=0.44$, $df=47$, $p=0.897$). Mass of hatchlings was significantly related to midline carapace length (SCL) ($t=14.22$, $df=82$, $p<0.0001$) but when mass was corrected for SCL there were no significant differences in mass among hatchlings of incubation treatments ($t=0.215$, $df=47$, $p=0.975$).

Table 3. Output from linear mixed-effect models of incubation duration (days) for painted turtle (*Chrysemys picta*) eggs incubated during the 2017 summer. Incubation treatments consist of auditory-early (A-E), auditory-late (A-L), control-early (C-E), C-L control-late (C-L), mechanical-early (M-E), mechanical-late (M-L) (see text for details). ($N_{individuals}$=151, N_{clutch}=20, N_{tubs}=20, N_{aud}=53, N_{mech}=50, N_{cont}=48, N_{early}=82, N_{late}=69). Bold values indicate significant differences.

Incubation Treatment	*B*	SE	df	t-value	p-value (corr)
A-E vs. C-E	0.32	0.4	46	0.81	0.97
A-E vs. M-E	0.86	0.37	82	2.31	0.20
A-E vs. A-L	1.81	0.61	17	2.97	0.08
A-E vs. C-L	**1.99**	**0.62**	**17**	**3.24**	**0.05**
A-E vs. M-L	**3.01**	**0.61**	**17**	**4.94**	**<0.05**
C-E vs. M-E	0.53	0.4	46	1.33	0.77
C-E vs. A-L	1.5	0.61	17	2.46	0.19
C-E vs. C-L	1.68	0.61	17	2.74	0.12
C-E vs. M-L	**2.7**	**0.61**	**17**	**4.45**	**<0.05**
M-E vs. A-L	0.96	0.62	17	1.55	0.64
M-E vs. C-L	1.14	0.62	17	1.83	0.47
M-E vs. M-L	**2.16**	**0.62**	**17**	**3.50**	**0.03**
A-L vs. C-L	0.18	0.44	46	0.41	1
A-L vs. M-L	**1.21**	**0.39**	**82**	**3.06**	**0.03**
C-L vs. M-L	1.02	0.44	46	2.32	0.21

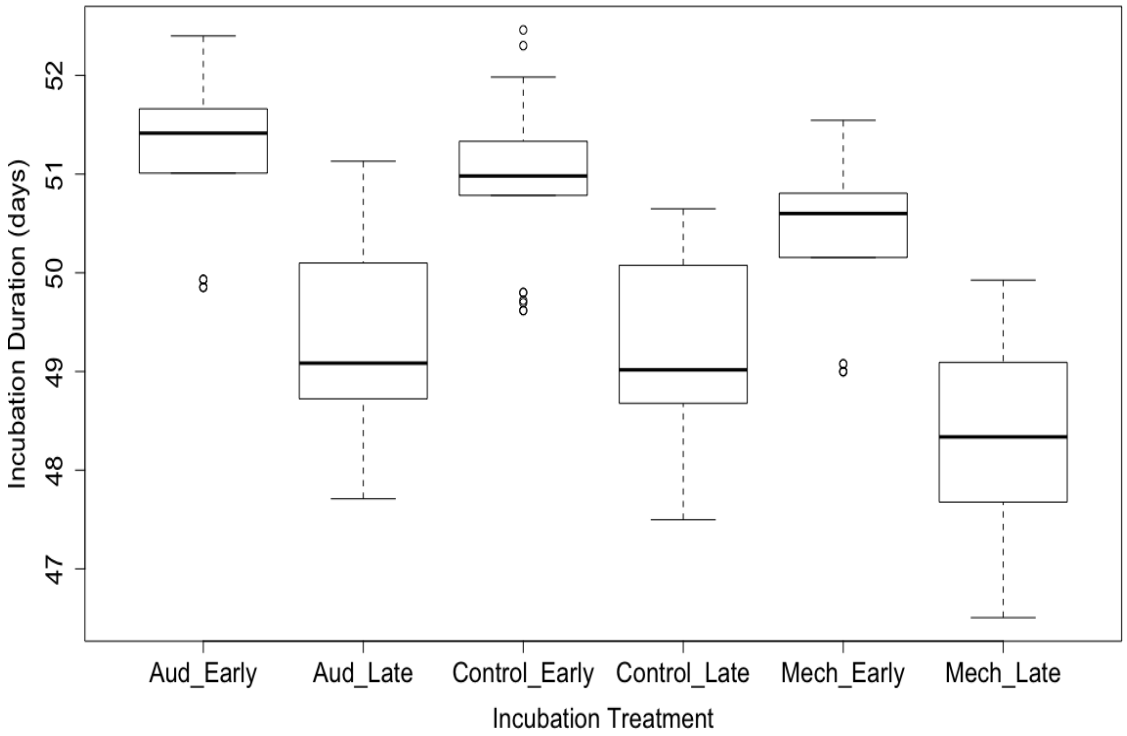

Figure 13. Box plots of incubation duration for treatment groups used in the linear mixed-effect models of painted turtle (*Chrysemys picta*) eggs incubated during the 2017 nesting season. Comparison of average incubation duration (days) among incubation treatments is shown for Early (day 1) and Late (day 4) groups, and their subdivided sections of mechanical communication (Mech), auditory communication (Aud), and control (Control). Incubation duration differed significantly among incubation duration treatments with the mechanical communication treatment having the shortest incubation, and mechanical-late being significantly faster than mechanical-early.

1.4 Discussion

Causes of Clustering

My study provides several lines of evidence to suggest that female painted turtles choose to nest together. Across multiple nesting seasons, females placed their nests in spatial and temporal clusters, suggesting that they may be participating in egg clustering or communal nesting. High nest density was not strongly associated with environmental characteristics of the site, as the only characteristics associated with nest location (slope and reduced understory vegetation) coincidentally occupy a large majority of the WHP embankment. Thus, good quality nesting habitat (suitable for proper embryonic development) is not limiting at WHP, such that environmental characteristics are important in a female's choice of general location, but are not a significant driver of precise nest location (Schwarzkopf and Brooks 1987). This is consistent with many species of communal nesting reptiles that cluster in areas where nesting sites are not limiting, therefore refuting the saturated habitat hypothesis (Doody et al. 2009). Not only did females nest in clusters, but when given options to nest with different densities of turtle models, they most often chose to nest near the highest density of models, strongly suggesting that females are using visual cues presented by other nesting females to choose their nest site. This also suggests that females may prefer to nest in a group or with other females, versus alone or farther from other nesting individuals. Doody et al. (2009) summarizes all cases of communal nesting in *Chelonia*, concluding that 16% of species have been observed nesting communally and Davis and Burghardt (2011) showed evidence to support that turtles can learn from visual cues and conspecifics. Vogt (pers. com.) also describes nesting aggregations of *Podocnemis expansa* that arrive and leave nesting areas together. It should be noted that my experiment took place during the second half of nesting season, which may have influenced my results if turtles

nesting at the start of the season are of different ages or experience levels. Iverson et al. (2016) found that female painted turtles nested more often near nests scented with urine versus water, supporting the idea that olfactory cues from other females are important in nest site selection. Although my experimental data found no strong evidence to suggest olfactory cues are important in nest detection, I did find plausible additional observational evidence to support this; many females (15-25%) in my study often placed their nests close to, or on top of, another female's nest, suggesting that females may be able to detect nests that have already been laid. This behaviour is most familiar and evident with sea turtle *arribada*, where in some locations thousands of females will nest on the same beach, and can dig up over 40% of other turtles' nests (Wen pers. com.). This also begs the question, is clustering a detrimental behaviour, as females are destroying other's nests? Could clustering just be a byproduct of density and only by chance females nest on each other's nests? Females may also be using scent to locate the nests underground as indicated by the "head-ploughing" or "ground-nuzzling" behaviour observed while females are searching for a nest location (Morjan and Valenzuela 2001; Iverson et al. 2016; Kell pers. obs.). My experimental setup may have placed treatments (scented chambers) too close together for turtles to distinguish and therefore ruled out a proper control, as Iverson et al. (2016) did find a difference with a distance 3x greater between treatments than mine. Turtle urine during our experiment was also mixed, which may have caused unforeseen negative influences on turtles.

Additional evidence to suggest that females choose to nest together comes from the observed peaks in nesting activity (Figure 3), which suggest that females are participating in nesting synchrony. Nesting synchrony has been observed in sea turtles (*Lepidochelys olivacea*; Eckrich and Owens 1995) and has been suggested in snapping turtles (Robinson

and Bider 1988). These nesting peaks seem to be related to weather. Nesting peaks tend to coincide with or occur shortly after warm rainfall (Burke et al. 1998; Spencer and Thompson 2003; Kell pers. obs.). Nesting during rainfall may improve the malleability of the soil, reducing the need for females to carry large amounts of liquid in their bladders, in turn reducing the risk of overheating, and covering scents used as cues by predators (Bowen and Janzen 2005; although rain has also been suggested to increase nest scent and therefore predation, Brooks unpub. data), and may act as a trigger to initiate nesting synchrony.

Benefits of Clustering

My study also suggests that females may be receiving a fitness benefit from nesting in clusters. Firstly, my data indicates that clustered nests may be able to contribute to higher survivorship than solitary nests, contrary to another study (Marchand et al. 2002). Clustered nests may cause prey saturation, supporting the attack abatement hypothesis (sensu Turner and Pitcher 1986) that predicts that predators are less likely to find a single group of many eggs than many scattered small groups. This hypothesis also encompasses a dilution effect: as the number of eggs increases, the potential for predation for each mother, neonate, or egg, is reduced (Doody et al. 2009). Prey saturation or predator swamping is often successful in the animal kingdom for reducing mortality (Sweeny and Vannote 1982). Red foxes (scent-oriented predator) and common ravens (visual-oriented predator), the two main predators at our sites, may be befuddled by clustered nests. This swamping strategy may be useful on predators that do not alter foraging behaviour after encountering nests (Wirsing et al. 2012). I found a nonsignificant 10% higher survivorship for the clustered nests at WHP during 2017, but with increased sample size this difference may become significant. Nest predation levels

are often naturally very high (Oddie et al. 2015), in some cases up to 100% (Congdon et al. 1987), so even a 10% increase in survivorship could contribute to increased recruitment and population growth over time.

Nesting synchrony is another form of predator swamping. Nesting synchrony would allow females to easily locate one another on the embankment (i.e., provide a strong visual cue), provide nest clustering opportunities, and reduce the search time needed on land to find an oviposition location (thus reducing their own risk of predation while terrestrial through reduced predator encounter time). Another reason for females to participate in egg clustering could be to further reduce this time spent on land during the excavation process. Nest excavation can take large amounts of time and energy, especially in areas of high soil compaction, so nesting in the same nest chamber or part of the same chamber as conspecifics, which many of my turtles did, could decrease this excavation time. Similarly, some species of snakes and lizards are known to deposit eggs in the same nest chambers as conspecifics (Rand 1968; Burger and Zappalorti 1991).

Another potential reason that turtles may cluster their nests relates to the egg insulation hypothesis, as communal egg-laying could provide a thermal advantage (Doody et al. 2009). Communal egg-laying in temperate amphibians has been shown to increase incubation temperatures and reduce incubation duration (Seale 1982; Waldman 1982; Hakansson and Loman 2004). Blouin-Demers et al. (2004) even suggest that a population of snakes bordering its northern range incurred a thermal advantage of nearly 5^{o}C by nesting communally. My study site borders the northern thermal limit for painted turtles (Ernst and Lovich 2009), so gaining a thermal advantage could be very beneficial.

Furthermore, I show evidence that through hatchling communication, hatchlings may be able to experience synchronized emergence by communication with embryos in neighboring nests. Synchronized emergence is another form of predator swamping and therefore also has the potential to increase fitness. I showed that the likely mode of communication for painted turtles is through mechanical stimulation (although chemical communication, such as carbon dioxide concentrations, were not tested and could have played a role), and this resulted in clutches laid at difference temporal periods to hatch within a close time period to their counterparts. My mechanical communication treatment had the shortest incubation duration, suggesting that through some form of vibrational or tactile stimuli, embryos can adjust their incubation rate to situate themselves at a similar developmental stage to their neighboring eggs, thus facilitating synchronous emergence (McGlashan et al. 2018). Other studies have shown that communication between turtle embryos is possible (Spencer et al. 2001, Colbert et al. 2010; McGlashan et al. 2012; McKenna 2016), and although the majority of papers suggest mechanical communication, auditory communication has also been proposed (Ferrara et al. 2013). Aubret et al. (2016a) even suggest that synchronization through embryonic communication may drive clustering behaviour in egg laying species. In addition to turtles (Doody et al. 2001; Spencer 2001), hatching synchrony has been seen in other taxa including invertebrates (Frechette and Coderre 2000), fishes (Bradbury et al. 2005), amphibians (Sih and Moore 1993), crocodilians (Ferguson 1985), squamates (Vitt 1991), and birds (Lack 1968; Schwagmeyer et al. 1991), through altering of incubation periods. Eggs that are at different developmental stages can speed up or slow down development to hatch at the same or similar time period; these rate changes are referred to as the “catch up” or “slow down” hypotheses (Spencer et al. 2001;

McGlashan et al. 2012). McGlashan et al. (2015) and Aubret et al. (2016b) suggest that heart beats may be used as the mechanism for communication, and suggest that embryos that are less developed can increase their heart rate to shorten their incubation period. One explanation suggested for why turtles possess embryo communication is to counter the lateral temperature gradient within a nest chamber that results in different developmental temperatures and times (Thompson 1989). Having the ability to alter developmental rate would allow cooler, less developed eggs, to speed up their development through metabolism and heart rate, allowing similar hatch times to their warmer siblings experiencing higher temperature variation (Thompson 1988, Brooks unpubl. data). Having coordinated emergence can allow the hatchlings to escape their nest chamber more easily (Spencer et al. 2001), or ensure optimal overwintering position in the nest for overwintering (McGlashan et al. 2018).

My study bridges a gap from within to between nest communication and synchrony, suggesting that it is possible for eggs from different clutches to communicate and therefore synchronize, as I have shown that females are selecting locations that would place their nests in close enough approximation, and eggs from different clutches can communicate and alter incubation duration. In addition to finding no evidence of a cost associated with synchronous hatching (no difference in body condition or yolk sac size between early and late hatchlings; supported by McGlashin et al. (2018) but contrary to Colbert et al. (2010)), hatching synchronously could also provide increased survivorship once the hatchlings leave the nest cavity. Clutches hatching synchronously may also experience higher survivorship in locations with avian predators. Spencer et al. (2001) found that Australian magpies (*Gymnorhina tibicen*) could usually only process one hatchling (*Emydura macquarii*) before

the rest of the clutch made it to the water. My study site is frequented by the common raven, an avian predator of similar size and intelligence to the magpie, and a frequent nest predator of painted turtles (Kell et al. unpubl. data), so similar predator handling events could take place. Tucker et al. (2007) suggested that for hatchling turtles, hatching synchrony may actually promote prey switching, such that nests that hatch earlier have a higher survivorship than later nests once predators recognize the new food source. Grouped eggs also hatch faster than solo eggs (McGlashan et al. 2015; Aubret et al. 2016b), therefore nests that can synchronize would theoretically hatch earlier than solitary nests, hatching earlier in the season before prey switching begins. Although we only show evidence that communication of hatchlings is possible and can affect incubation times suggesting synchronized emergence is plausible, the fitness benefit of this is only theoretical. Females may also participate in nest clustering due to a kin selection hypothesis, suggesting that relatives from the same maternal lineage are expected to aggregate to the same laying area and contribute to nest clustering (Tallamy 1985), but further research on this topic needs to be investigated at this site.

In conclusion, I show evidence that female painted turtles choose, and may want to choose nesting locations near nests of other females. I also show evidence to suggest that female painted turtles can locate nests based on visual cues provided by other females and the olfactory cues from nests. This clustering behaviour may be driven by the benefits that the nests receive before and after hatching, and seems to be based in predator swamping or prey saturation as my data suggest that nest clustering may increase the survivorship of nests. My study is also consistent with others suggesting that communication among turtle eggs is possible, and may allow embryos, whether from the same clutch, or from different clutches in close proximity, to synchronize hatching.

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Conclusion

Turtle populations across the globe are declining at alarming rates (Gibbons et al. 2000; van Dijk et al. 2014; Lovich et al. 2018). Yet despite this decline and the vast amount of research already conducted, there is still so much that we do not know about turtle biology that could be crucial to their recovery. It is almost puerile to think that up until now turtles have been considered a group that is not surprisingly slow, but also non-social and to an extent brainless (Wilson 1975). However, new research is shedding light into this area, and reveals that turtles are indeed more complex than was previously thought.

My study focussed on this topic of social behaviours in turtles, and investigated behaviours that have been observed in our long-term study population. My data suggest that indeed, turtles in our population are participating in nest clustering and communal nesting, while also participating in nesting synchrony, and that females are using visual and potentially olfactory cues from other females to help in the nest site selection process. I also found some evidence to suggest that this clustering behaviour is benefiting females by increasing survivorship of nests, likely through a form of predator swamping. Lastly I show evidence to suggest that turtle embryos can communicate during development, thereby altering their incubation duration, which could permit synchronized emergence, thus also resulting in predator swamping at hatching.

These results support several hypotheses proposed to explain why egg-laying species cluster nests. Firstly, nest clustering may result from nesting habitats being limiting, as stated by the saturated habitat hypothesis (Doody et al. 2009). My data do not seem to support this hypothesis because in our population there is more suitable nesting habitat than is occupied. Secondly, the attack abatement hypothesis predicts that groups of eggs are less likely to be

found by predators than many spread out eggs (sensu Turner and Pitcher 1986). I showed evidence for this hypothesis by demonstrating that clustered eggs had higher survivorship than solitary eggs. A "scent saturation" strategy may also be possible here, as nests may become camouflaged by surrounding predated nests once scent (i.e., from eggshells and sand) are scattered over top of neighboring intact nests, and visually by looking non-rewarding to predators, as the open hole for the predated nests may mask the presence of the intact nest. Thirdly, I provided evidence to support the maternal benefit hypothesis which states that communal egg-laying may provide maternal benefits by saving time and energy, or by increasing the chance of survival (Rand and Dugan 1983; Rauch 1988). Behaviours such as nesting synchrony and nesting in the same nest chamber as conspecifics can reduce both time and energy for nesting females; both of these behaviours were observed in my study.

Nesting synchrony may be best explained by the weather patterns surrounding nesting season. In my study population, the number of nesting peaks usually coincides with the number of clutches females can produce during a given season (2 clutches/year at my site; Brooks unpubl. data), but the magnitude and timing of these peaks are driven by environmental conditions. Nesting peaks tend to be triggered by warm rainfall (Burke et al. 1998; Spencer and Thompson 2003; Kell unpubl. data. Previous fall and current spring temperatures also affect the size of these peaks, because these seasonal temperatures affect the amount of energy a female can store and invest into reproduction (Rollinson et al. 2012). Warm fall temperatures and cool spring temperatures seem to be the optimal conditions for female reproduction, as they provide optimal energy storage and allocation (Hendrick et al. 2017).

Nest clustering has the potential to reduce excavation time by females, and to provide a fitness benefit. For females to maximize the reduction in excavation time and energy, they should nest directly in the nest cavity of another female (Rauch 1988). Using a conspecific's nest chamber often results in the destruction of those first female's eggs and may not offer any additional protection to the second female's nest. Females nesting directly in conspecifics nest chambers would therefore only benefit themselves if they destroy the first nest. Nest destruction by conspecifics happened in about 30% of the clustered nests I observed. More frequently, nests were clustered without harm to each other. This clustering may benefit both female's nests. Aubret et al. (2016b) found evidence that hatchlings snakes from eggs incubated in clusters were more social than eggs incubated alone. Nest clustering could benefit both females overall and a female's fitness could also be further increased if she nests with related individuals, as suggested by the kin selection hypothesis. Nesting in a cluster of nests with related embryos would not only increase the chance that a female's nest may survive through predator swamping, but if her nest is predated, she may still accrue a fitness benefit (albeit, a reduced benefit) if the related embryos survive. The "head-plowing" behaviour observed during nest-searching could also then in addition to being used for detecting the location of nests, could be used for detecting the relatedness of the individual's nest. Kin recognition through proteins in the urine is seen in mammals and fish (Moore et al. 1994; Sherborne et al. 2007), and should be investigated in turtles.

It is clear that there is still a large knowledge gap about turtle nesting behaviour and ecology, and it is important that we continue to study these topics. Gathering new information about species can be challenging, especially for species that are cryptic, occur at low density, or are declining, such that any interference may damage populations (Kellogg

and Shaffer 1993). The use of model organisms, such as the painted turtle (*Chrysemys picta*), can significantly help this problem. Model organisms are usually available in high numbers and their biology is supported with more literature, so studying them is easier. This is why novel findings in well-studied organisms, as is the case with my study, are so important. It is essential that we continue to study these organisms and areas of science, to increase our knowledge of biology and evolution, but also consequently, conservation.

My research may help in the conservation of turtle species. Creation of artificial nesting areas for turtles is an under-researched method, but is often implemented to prevent females from nesting in dangerous locations such as roadway shoulders (Paterson et al. 2013). Transplanting nests to the mounds (Paterson et al. 2013) or putting model turtles (my study) on new mounds may help make nesting mounds more desirable to females, guiding them away from dangerous sites. My study may also help to understand what affects nesting along road shoulders have on turtles. Compared to natural nesting sites, road shoulders provide abundant locations to choose for nesting. Abundant choices could increase turtle search time if they are searching for other females or nests, or could have consequences if turtles prefer to nest together and cannot find conspecifics. Turtle social behaviour, both inside and outside of nesting, is fascinating and starting to reveal a more in-depth story. Research needs to continue into these areas to provide more key answers and windows into turtle biology, ecology and conservation.

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